Phytase From Transgenic Alfalfa

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Introduction

Buildup of phosphorus in the environment and the resulting degradation of water resources is of mounting concern. Much of this buildup is traceable to human activities. Important among these is livestock production. Monogastric animals, such as poultry and swine which can solubilize only a small fraction of the phosphorus in their grain-based rations, while excreting the remainder, have come under increased scrutiny. Supplementation of inorganic phosphorus into rations to meet animal nutritional requirements exacerbates the problem.

Much of the phosphorus in grain is in the form of insoluble phytates. Researchers have shown that supplementing poultry and swine rations with the enzyme phytase can lead to solubilization of the phosphorus, thus eliminating the need for phosphorus supplementation and concurrently reducing the level of phosphorus in the excrement to approximately one-half of that normally experienced.

Because of relatively higher population and animal densities in western Europe, concern over phosphorus buildup has preceded that in the U.S. Accordingly, certain areas, like the Netherlands, have mandated limits on animal numbers and/or required the use of phytase in animal rations.

The enzyme phytase derived from *Aspergillus niger* has, to date, generally been produced in fermentation vats using genetically engineered microorganisms. It has been estimated that the cost of phytase supplementation with this material would be about three times the cost of conventional supplementation with dicalcium phosphate.

As an approach to reducing the cost of phytase production, a multi-disciplinary ARS-UW team at Madison, Wisconsin has produced transgenic alfalfa with the capability of expressing phytase. This phytase can be recovered from juice extracted from the herbage. Other constituents of the juice including xanthophyll, used to pigment egg yolks and broiler skin, high levels of dietary protein, and various vitamins and minerals add to its value in rations. The use of whole alfalfa herbage, however, would not be desirable due to its high fiber content. Since phytase would potentially be needed in great quantities, but not in very pure or concentrated form, it is believed that the economic advantage of production in "plant bioreactors" such as alfalfa would be great. The advantage in capital costs is particularly great. Ideally, the cost of phytase supplementation should be competitive with the traditional dicalcium phosphate supplement, with the environmental benefits as an added incentive.

Methods

Sixteen phytase-producing alfalfa transformants were originally created. Early bioassays indicated that these transformants produced phytase at a range of levels. These transformants were vegetatively propagated in the greenhouse during the winter and spring of 1997 and approximately 7500 plants were set out into the field in mid-May in both replicated research plots as well as larger "production plots." The plant densities in the two plot types were 9680 and 13936 per acre, respectively. The plots were harvested on August 1 and September 15. Concurrent with this work, transformants were crossed with elite production strains to produce plants which will be repeatedly back-crossed until seed is produced which will yield approximately 95% phytase producers which have desirable persistence and production characteristics.

Results

The transformants generally did well under field conditions. The August 1 harvest was affected by rather severe insect damage. Insects were subsequently controlled by spraying.

Phytase concentrations of the best six transformants ranged from approximately 0.85% to 1.8% of soluble protein and from approximately 21,000 to 36,500

phytase activity units per liter of juice expressed. Suggested activity units per ton of feed are 400,000 and 1,200,000 for poultry and swine, respectively. Therefore, the ranges of juice product required per ton of feed would be from 11-19 liters for poultry and 33-57 liters for swine. The per acre-year anticipated yields and values are shown in Table 1 for five high-yielding transformants. A value of $1.50 per ton of feed is arbitrarily assigned. This is approximately half of the current cost of supplementation with dicalcium-phosphate.

Conclusion

Alfalfa plants vegetatively propagated from the original transformants performed well in the field producing phytase at economic levels. Field work will continue in 1998 with plants produced from seed.

Table 1. Quantity and value of phytase produced in five alfalfa transformants.

Plant No.	Activity units/ Liter juice	Activity units/acre (1 cutting)	Activity units/ acre-year (20 ton fresh wt)	Tons poultry feed/acre-yr @ .4 x 10^6 units/ton	Value/acre-yr in poultry feed @$1.50/ton	Tons swine feed/acre-yr @ 1.2 x 10^6 units/ton	Value/acre-yr in swine feed @ $1.50/ton
360	36,500	37.7 x 10^6	282 x 10^6	705	$1056	235	$353
410	30,000	24.2 x 10^6	264 x 10^6	660	990	220	330
472	29,000	32.4 x 10^6	261 x 10^6	653	978	218	327
411	23,000	30.6 x 10^6	197 x 10^6	493	740	164	246
420	21,000	24.0 x 10^6	161 x 10^6	403	605	134	201